the application. The foregoing amendments and following remarks are responsive to the non-final Office Action mailed April 26, 2000.

Applicants have made two grammatical corrections in claim 1, changing "*in vivo*" and "exogenously added" into proper adjectives by inserting hyphens. In addition, to be consistent with the amendment to claim 1 in the previous response, and to provide correct antecedent basis in dependent claims, the term "turnover" had been changed to "deadenylation and degradation" in the other claims, as supported, for example, on page 15, lines 22-23 of the Specification.

Claim Rejections - 35 U.S.C. § 112, Second Paragraph

Claims 21-45 have been rejected for reciting a method for identifying an agent capable of modulating the *in-vivo* stability of a target mRNA sequence. The Examiner asserts there is no support other than for *in-vitro* modulation.

Applicants respectfully request reconsideration of the rejection. Applicants' invention is directed to a system and methods for recapitulating *regulated* RNA deadenylation and degradation, regulated RNA deadenylation and degradation being a feature of *in-vivo* RNA deadenylation and degradation. Applicants have for the first time devised a system in which such regulated deadenylation and degradation occurs without artifactual RNA degradation. As noted in the Background section on page 3, lines 16-20, "[a] significant difficulty in the development of these systems is to differentiate between random, non-specific RNA degradation and true, regulated mRNA turnover. The significance of all previous *in vitro* systems to the true *in vivo* process of mRNA stability, therefore, is unclear. To date, no *in vitro* mRNA stability system has been generally accepted in the field as valid and useful." And on page 41, lines 10-13, it is stated that "[m]oreover, the *in vitro* system of the invention has ready applications in high throughput assays to screen libraries of compounds to elucidate which compounds may have applications as pharmaceuticals which can modulate the stability and turnover of RNA transcripts *in vivo*, and

thus be used to treat a wide variety of disease or disorders." In addition, on page 35, lines 6-11, it is pointed out that the aforedescribed method may be used to identify compounds capable of intervening in cellular transformation or immune dysregulation. As Applicants' invention is directed to a method for recapitulating *regulated* RNA turnover (deadenylation and degradation), regulation being an *in-vivo* phenomenon, Applicants believe that adequate support is provided for identifying agents capable of modulating *in-vivo* stability, and withdrawal of the rejection is respectfully requested.

Claim Rejections - 35 U.S.C. § 112, Second Paragraph

Claim 38 has been rejected under 35 U.S.C. § 112, second paragraph, for lack of antecedent basis for the term "said labeled target RNA."

Applicants traverse the rejection, the foregoing amendment correcting the dependency of claim 38 to claim 36, in which the term is recited. Withdrawal of the rejection is requested.

Claim Rejections - 35 U.S.C. § 102

Claims 1-2, 8-10, 12-15, 21, 24-30 and 51-52 have been rejected under 35 U.S.C. § 102(b) as being anticipated by Bernstein. The Examiner indicates that the features on which Applicants have relied in the previous response regarding the cell extract, not containing nuclei and nuclear contents and ribosomes excluded by centrifugation at 100,000 x g, are not present in the claims.

Applicants respectfully traverse the rejection. Bernstein's system has nothing in common with the system of the instant invention. Bernstein et al. employ a system based on a cell-free protein translation assay, to which is added a cell-derived extract, "ribosomal salt wash" ("RSW"), entirely different from that of the instant inventors. RSW is prepared by *isolating* polysomes (ribosomes) from homogenized cells, suspending the isolated polysomes in a high-salt (0.3 M KCl) buffer to extract polysome components, and subsequently removing the polysomes

by centrifugation and using the supernatant, RSW, in the assay. RSW, and hence Bernstein's system, therefore *excludes* soluble substances from the cytoplasm and *includes* materials removed from the ribosomes under high salt conditions. In contrast, the instant inventors' cytoplasmic extract is a supernatant from a 100,000 x g, 1 hour centrifugation which is *depleted* of polysomes ("S100"). The instant system *includes* cytoplasmic substances and *excludes* materials removable from polysomes under high-salt conditions; and moreover, by way of the foregoing amendment, includes the feature that the cytoplasmic extract is depleted of activity of proteins that bind polyadenylate. Accordingly, by way of amendment, the subject matter of (previously amended) claim 7 with regard to the cell extract being a 100,000 x g, 1 hour cytoplasmic extract, has been incorporated into claim 1. Applicants believe that this amendment overcomes the rejection as the claim no longer reads on Bernstein.

The Examiner also has asserted that Applicants' previous amendment to claim 1 replacing "turnover" with "deadenylation and degradation" reads on Bernstein because Bernstein states that "...mRNA decay intermediates were observed in reactions...one...class corresponded to mRNA molecules whose poly(A) tracts were being shortened....[t]he second...migrated...at the same position as deadenylated mRNA...These intermediates suggest a stepwise decay pathway in which the poly(A) is first shortened and then removed and...the deadenylated mRNA is then completely destroyed..."

Applicants respectfully traverse the rejection. Applicants point out that, whereas the instant system is capable of recapitulating *regulated* mRNA deadenylation and degradation, wherein the poly(A) is specifically and sequentially removed by a poly(A)-specific nuclease (i.e., deadenylase), and subsequently degraded, Bernstein does not achieve such a regulated mRNA deadenylation and degradation. No evidence is provided that regulated deadenylation and degradation of mRNA is occurring in the Bernstein system. Bernstein points to the experiment

described in Figure 9 to demonstrate shortening of the poly(A) tract, but no convincing evidence is present to assure that degradation shown therein mimics the *in-vivo* process. Applicants clearly demonstrate a stepwise decay pattern in Figure 1 of the instant application; Bernstein's Figure 9A shows a smear pattern from which it is not possible to discern regulated deadenylation in contrast to the effect of known, non-specific 3' to 5' exonucleases which may be responsible for cleaving the poly(A) in the pattern demonstrated. In fact, subsequent workers using the RSW system of Bernstein (mentioned above) indeed have shown that a specific 3' to 5' deadenylase (i.e., that responsible for regulated RNA deadenylation) is not stably associated with polysomes or ribosomal subunits (Korner et al., 1998, *EMBO Journal* 17:5427-37), and thus, RSW does not contain any components which can contribute to regulated RNA deadenylation. This albeit-later appreciation of the components (or lack thereof) in the RSW-containing system of Bernstein et al. and its inability to recapitulate regulated RNA deadenylation and degradation is evident in observations and statements from the same laboratory where the Bernstein work was conducted. Additional studies on the Bernstein system using RSW (Brewer and Ross, 1988, *Mol. Cell. Biol.* 8:1697-1708; document AF in the Information Disclosure Statement submitted April 28, 2000) questions what nucleases are responsible for the deadenylation and subsequent degradation steps ("We do not know what types of nucleases are responsible for either step." [page 1704, left column, lines 8-9]). Furthermore, the link between deadenylation and degradation is questioned as a result of the Brewer and Ross work: "[p]erhaps the major question raised by our experiments concerns the link between poly(A) removal and mRNA degradation." (Page 1704, left column, lines 10-12), and a conclusion that "...therefore, cannot say whether it is deadenylated before being degraded." (Page 1704, left column, line 18-19).

Therefore, it is clear that the Bernstein et al. observations in light of contemporaneous work from the same laboratory and later work of others that Bernstein does not demonstrate

regulated deadenylation and degradation. In light of the above and foregoing, withdrawal of the rejection is respectfully solicited.

Claims 1, 4-6, 12, 14, 16-17, 21-25, 28 and 55 have been rejected under 35 U.S.C. 102(b) as being anticipated by Krikorian. The Examiner asserts that because deadenylation is an inherent property of mRNA degradation as evidenced by the teachings of, for example, Bernstein, where deadenylation precedes degradation, Krikorian is examining degradation/decay and therefore, necessarily performs deadenylation of the RNA. The Examiner also points out that while Krikorian uses infected cells as the source of the extract and differs from the claimed invention, the claims are drawn to cells that are either infected or uninfected. Furthermore, the Examiner points out that Krikorian uses an RNA from an exogenous source.

Applicants respectfully traverse the rejection. Krikorian's experiments used a cytoplasmic extract prepared from a low-speed spin of a cell lysate, and did not utilize a 100,000 x g, 1 hour cytoplasmic extract isolated from eukaryotic cells or tissues, in which the extract is depleted of activity of proteins that bind polyadenylate, as is now the focus of the claims. Thus, *regulated* RNA deadenylation and degradation of a preselected RNA was not achieved; and therefore, the claims as amended do not read on the teachings of Krikorian. Moreover, Applicants submit that the features of the instant claims different and distinct from the prior art are not dependent on the particular source of exogenous RNA added to the system, which may be from any source, including a source foreign to or the same as the host cell from which the cytoplasmic extract is prepared. It is noted that on page 50, lines 13-14, in a description of the instant assay system, "[t]his property affords variety in RNA substrate preparation and sequence manipulation." In light of the foregoing, withdrawal of the rejection is therefore courteously requested.

Claim Rejections - 35 U.S.C. § 103

Claim 46 has been rejected under 35 U.S.C. § 103(a) as being unpatentable over Bernstein in view of Brewer and in further view of Krikorian. The Examiner asserts that it would be obvious to one of ordinary skill in the art at the time the invention was made to utilize a system of evaluating mRNA decay in the presence of a nucleotide triphosphate, then introduce an agent to evaluate the ensuing effects on the adenylation of target RNA because the system of Bernstein was successful at evaluating exogenously introduced agents, and observed their effects on mRNA stability, and that deadenylation precedes degradation. The Examiner goes on to point out that Brewer teaches a system that monitors deadenylation of mRNA and that poly(A) shortening precedes degradation of mRNA with AU-rich sequences; Krikorian demonstrated the use of ATP, GTP, etc., in the performance and evaluation of mRNA deadenylation and degradation in his system.

Applicants request reconsideration of the rejection. Applicants believe that the foregoing amendment to claim 46, in which the claim has been written in independent form to include the components relating to deadenylation present in claim 1, render the claim non-obvious in light of the prior art. As noted above, regulated RNA turnover, comprising deadenylation and degradation, and particularly the first step of regulated (i.e., *in-vivo*) deadenylation, heretofore had not been achieved in an *in-vitro* system, because previous investigators had not prepared the appropriate cell extract to *include* the required deadenylase enzyme, and to *exclude* components which non-specifically cleave poly(A) and/or disrupt or inhibit regulated deadenylation and degradation. The instant inventors have achieved a system which recapitulates regulated deadenylation and degradation, of which regulated deadenylation precedes degradation. The cytoplasmic extract depleted of activity of proteins that bind polyadenylate includes the required enzyme and excludes inhibitors; RSW, as mentioned above, includes non-specific exonucleases

and other components. In light of the foregoing, withdrawal of the rejection is requested.

Claims 31-32 and 44-45 have been rejected under 35 U.S.C. 103(a) in view of Bernstein in view of Chen and in further view of any one of Zhang, Myer, Nakagawa, Levine, Nagy, Nakamaki, or Liu. The Examiner asserts (from a previous Office Action) that because Bernstein demonstrates an in vitro system for evaluating mRNA stability, and the other investigators describe factors which modulate mRNA decay; that one of ordinary skill in the art would be motivated to evaluate the effects of additional ARE binding proteins because the system had been used by Bernstein to evaluate the effects of one such protein.

Applicants request reconsideration of the rejection. As discussed above, Applicants believe that the foregoing amendment to claim 1, on which claim 21 and thus the rejected claims depend, and in which the assay components which permit regulated RNA deadenylation and degradation are provided, provides a non-obvious distinction from the prior art set forth by the Examiner. As no one previous to the instant inventors had managed to provide a system capable of recapitulating regulated (i.e., *in-vivo*) RNA deadenylation and degradation, and, as set forth by previous amendment to claim 21 as "in-vivo stability," the ability of agents to modulate the *in-vivo* stability was not previously determinable. In light of the above and foregoing, withdrawal of the rejection is requested.

Claim 47 has been rejected under 35 U.S.C. § 103(a) as being unpatentable over Bernstein in view of Brewer and in further view Krikorian. In a previous Office Action, the Examiner pointed out that Bernstein demonstrates a system and method for evaluating the effect of exogenously added agents (PABP) on the stability of poly(A) mRNAs, a system comprising target RNA and cell extract depleted of poly(A) binding protein is utilized wherein exogenous PABP is introduced into the system and the effect of PABP on the resulting degradation of mRNA is evaluated. The Examiner acknowledged that Bernstein does not teach the monitoring

of deadenylation and degradation of target RNA and does not explicitly teach the relationship between deadenylation and degradation of target mRNA. The Examiner asserts that Brewer demonstrates a system and method for monitoring deadenylation and degradation of target RNA and teaches that poly(A) shortening precedes degradation of mRNA with AU-rich sequences at the 3' end, but Brewer does not specifically teach the addition of nucleotide triphosphate to the system of mRNA deadenylation and degradation. The Examiner pointed out that Krikorian demonstrates the use of an in vitro mRNA degradation system, the system comprising target mRNA and cell extract: in an experiment to determine whether virion host shutoff-induced in vitro mRNA degradation was dependent upon the components of an energy- generating system, parallel in vitro degradation experiments were conducted in which half of the reactions contained all of the components of the standard reaction, including ATP, GTP, etc, and the other half did not contain these elements. The degradation of mRNA was then observed. It is the Examiner's opinion that one of ordinary skill in the art would have been motivated to utilize a system of evaluating mRNA decay in the presence of a nucleotide triphosphate, then introduce an agent to evaluate the ensuing effects on the deadenylation and degradation on the target RNA because Brewer taught that polyadenylation of the mRNA precedes degradation of the RNA and Bernstein had earlier described and utilized a system of evaluating exogenously introduced agents introduced into the system and observing the resulting effect on mRNA stability by monitoring the degradation of the mRNA.

Applicants request reconsideration of the rejection. By way of the amendment to claim 47 to focus the claim on regulated deadenylation and degradation of a target mRNA molecule, and the previously-described amendment to claim 1, on which claim 47 depends, Applicants submit that a distinction has been provided which overcomes any obviousness issues with respect to the combination of the citations. As noted previously, no one had previous successfully

demonstrated regulated RNA deadenylated and degradation in an in-vitro system, and this claim provides a method heretofore unachievable and unobvious over any combination of the prior art.

Withdrawal of the rejection is therefore courteously solicited.

Claim 53 and 54 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over Bernstein in view of Krikorian. As noted in a previous Office Action, the Examiner indicated that Bernstein utilized a system in which poly(A) mRNA stability was evaluated: cell extract was depleted of poly(A) binding protein since RNA binding proteins that bind to ARE of mRNA was known to affect the stability of the mRNAs. It is the Examiner's opinion that one of ordinary skill in the art would have been motivated to package the cell extract and reagents of Bernstein into a kit because it was well-known and common knowledge in the art to package together reagents into a kit to facilitate practice of methods requiring said agents.

Applicants respectfully traverse the rejection. By way of the foregoing amendment to claim 53, in which the nature of the cytoplasmic extract is described as a supernatant of a 100,000 x g, 1 hour centrifugation, and is depleted of activity of proteins that bind polyadenlyate, a non-obvious distinction over the cited prior art is provided. As noted above, the amendments provide a system heretofore unachievable which recapitulates regulated RNA deadenylation and degradation. In light of the foregoing, withdrawal of the rejection is requested.

With regard to the Examiner's final comment regarding support for the term "*in-vivo*" now present in claims 21 and 33 by previous amendment, Applicants submit that adequate support is provided thereto by the Specification as noted above, in particular the discussion in the Background section which describes the unclarity of the relationship of all previous *in-vitro* systems to the "true *in-vivo* process of mRNA stability"(emphasis added), and that to date, no *in-vitro* mRNA stability system has been generally accepted in the field as valid and useful. Thus, Applicants and as such the use of these terms is not believed to represent new matter.

Withdrawal of the rejection is courteously requested.

Fees

A check in the amount of $485.00 is enclosed for a three-month extension of time and one dependent claim changed to an independent claim. No other fees are believed to be necessitated by the instant response. However, should this be in error, authorization is hereby given to charge Deposit Account No. 11-1153 for any underpayment, or to credit any overpayments.

CONCLUSION

Applicants respectfully request entry of the foregoing amendments and remarks in the file history of the instant Application. The Claims as amended are believed to be in condition for allowance, and reconsideration and withdrawal of all of the outstanding rejections is therefore believed in order. Early and favorable action on the claims is earnestly solicited. Should a telephone discussion assist in the prosecution of the application, the Examiner is invited to call the undersigned at (201) 487-5800, ext. 103, to effect a resolution.

Respectfully submitted,

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